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Expression of *Escherichia coli* Heat-labile Enterotoxin B Subunit (LTB) in *Saccharomyces cerevisiae*

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Heat-labile enterotoxin B subunit (LTB) of enterotoxigenic *Escherichia coli* (ETEC) is both a strong mucosal adjuvant and immunogen. It is a subunit vaccine candidate to be used against ETEC-induced diarrhea. It has already been expressed in several bacterial and plant systems. In order to construct yeast expressing vector for the LTB protein, the *elt*B gene encoding LTB was amplified from a human origin enterotoxigenic *E. coli* DNA by PCR. The expression plasmid pLTB83 was constructed by inserting the *elt*B gene into the pYES2 shuttle vector immediately downstream of the *GAL1* promoter. The recombinant vector was transformed into *S. cerevisiae* and was then induced by galactose. The LTB protein was detected in the total soluble protein of the yeast by SDS-PAGE analysis. Quantitative ELISA showed that the maximum amount of LTB protein expressed in the yeast was approximately 1.9% of the total soluble protein. Immunoblotting analysis showed the yeast-derived LTB protein was antigenically indistinguishable from bacterial LTB protein. Since the whole-recombinant yeast has been introduced as a new vaccine formulation the expression of LTB in *S. cerevisiae* can offer an inexpensive yet effective strategy to protect against ETEC, especially in developing countries where it is needed most.

Key words: enterotoxigenic *Escherichia coli,* LTB, *Saccharomyces cerevisiae*, gene expression, immunoblotting

ETEC is the most common cause of diarrhea, especially in young children, travelers and military personnel in developing countries (Katz *et al.*, 2003; Steinsland *et al.*, 2003). The major disease agent of ETEC is the heat-labile enterotoxin (LT). The LT is a plasmid-encoded, high-molecular weight toxin, which is immunologically and physicochemically related to cholera toxin (CT) (Guidry *et al.*, 1997; Fleckenstein *et al.*, 2000).

The crystal structure of LT revealed that it is composed of one A subunit (LTA) (27 kDa) and five non-covalently associated B subunits (LTB) (11.6 kDa each) forming a ring-like pentamer. LTA has ADP-ribosilating activity that causes constitutive activation of adenylate cyclase, an increase in the intracellular cAMP and subsequent severe diarrhea (Nataro and Kaper, 1998; Kozuka *et al.*, 2000). LTB is able to bind to ganglioside GM1 [Gal(β1-3)GalNAc(β1-4)(NeuAc(α2-3))Gal(β1-4)Glc(β1-1)ceramide], a glycosphingolipid found ubiquitously on the cell membranes of mammals and to other related receptors, such as GD1b-ganglioside, asialo-GM1, lactosylceramide and certain galactoproteins (Williams *et al.*, 1999). The LT and its related cholera toxin (CT) are extremely potent immunogens following mucosal or systemic delivery.

It has been shown that LT acts as a strong mucosal adjuvant, which enhances serum and local immune responses to co-administered antigens, where most antigens are unable to induce immune responses. Therefore, it is not surprising that LT has been incorporated into putative mucosal vaccines to guard against a range of infectious agents. However, its inherent toxicity and allergenicity have hampered progress for human use (Williams *et al.*, 1999).

One approach to overcome these problems is the use of a non-toxic derivative of LT, like LTB in isolation. Several studies with animal models and one human trial demonstrated that recombinant LTB (rLTB) can stimulate strong serum and mucosal immune responses against LT. Many studies have indicated that LTB could be used as a potent adjuvant (Tochikubo and Yasuda, 2000). Other investigations have also suggested that the rLTB can increase tolerance to heterologous antigens, a finding that has led to its further application in attempts to prevent autoimmune

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